Photosystem I of Chlamydomonas reinhardtii Contains Nine Light-harvesting Complexes (Lhca) Located on One Side of the Core

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SUPPLEMENTAL INFORMATION

Figure S1. SDS-PAGE analysis of the gradient bands obtained from the "flow-though" fraction of the Ni(II)-column

The presence of two PSI supercomplexes with different size in the sucrose gradient following affinity chromatography suggested that part of the antenna has been lost during purification. To test this hypothesis, the fraction that did not bind to the column ("flow-through') was loaded on a sucrose density gradient, and the green bands (not shown) were harvested and analysed by SDS-PAGE. Lhca polypeptides were visible in the upper bands, along with antenna complexes of PSII (Lhcb), indicating that indeed some of the Lhca complexes are lost during purification.

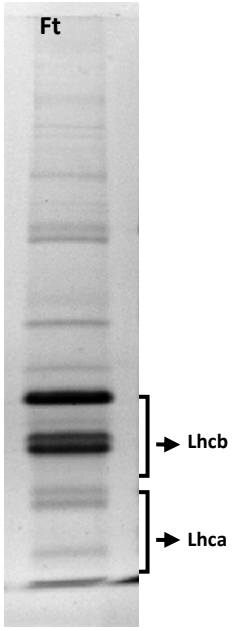

Figure S2. Electron microscopy of PSI-LHCI

Electron micrograph of negatively-stained *C.r.*PSI-LHCI, demonstrating the homogeneity of the preparation. Scale bar = 100 nm.

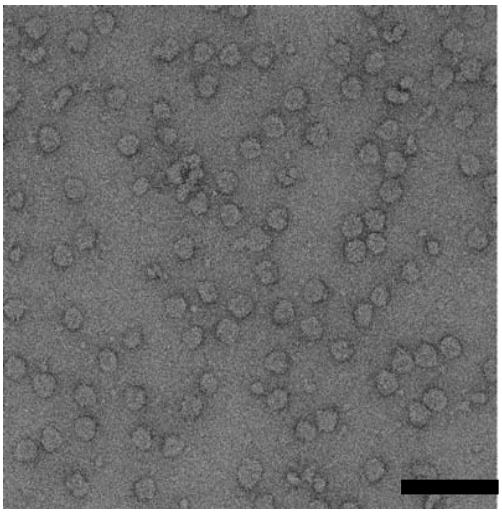

Figure S3. Analysis of the EM projection of PSI-LHCI and comparison with plant supercomplexes

The EM projection maps of *C.r.*PSI-LHCI (**A,B**) and *A.t.*PSI-LHCI-LHCII supercomplexes (**C**, adapted from Kouřil et al. 2005) and a projection map of pea PSI-LHCI complex (**D**) viewed from the stromal side and generated from the atomic model (Amunts et al. 2010) by truncating to 15-Å resolution using routines from the EMAN package (Ludtke et al. 1999). The green contours mark the strongest density of the PSI core complex. The yellow arrow in **D** indicates the strong density of the C helix of Lhca2 subunit, which was used as a marker for the assignment of the high-density spots of the outer Lhca half-ring in the *C.r.*PSI-LHCI supercomplex (see Figure 7). The C helix is almost perpendicular to the membrane plane and separated from the other two helices. Under such circumstances staining molecules can optimally accumulate around this helix and a projection of such a helix will result in a clear, strong density spot. Yellow arrows in (A) indicate high densities resolved in the outer row of Lhca, which is attributed to the C helix of Lhca protein in (the one of Lhca2 is indicated in D).

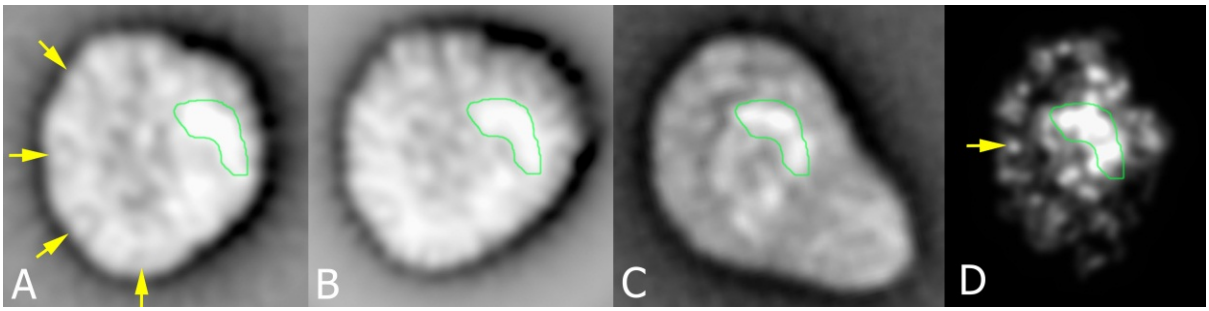

Figure S4. Analysis of the EM projection of PSI-LHCI and comparison of the two projections

Comparison between the projection maps of two main classes of particles observed in the PSI-LHCI fraction. A and B are the same as in Fig. 6. The contour of the particles and some internal high density areas have been marked in green and red. The overlay of the two profiles (C) shows that the positions of the high-density areas are very similar, indicating that the differences between the two particles are not caused by a different tilting of the same particle on the carbon support film used for EM specimen preparation. Hence, the difference in density is caused by the absence of a protein subunit in B compared to A. The extra area observed in A has a width of around 15 Å, and it is thus too small to accommodate an Lhca complex. It is suggested that the extra density represents PsaH and/or PsaL subunits in agreement with the biochemical data that shows that these subunits are easily lost during purification. Scale bar is 10 nm.

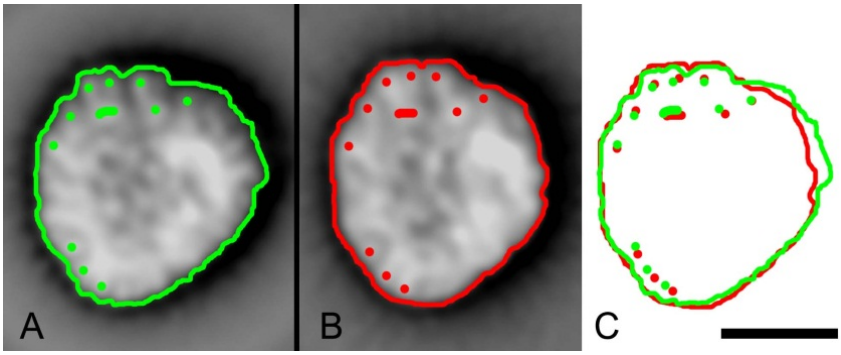

Table S1. Peptide identification table for the PSI-LHCI complex (fraction A1)

Protein	Protein accession numbers	Protein MW (Da)	# of unique tryptic peptide	# of total spectra	Peptide sequence	Best Mascot Ion score	Best Mascot Identity score	# of total spectra	Calculated +1H Peptide Mass (AMU)
Lhca1	tr|Q05093|	23,883	11	63	AGNWLPGSDAPAWLPDDLPGNYGFDPLSLGKEPASLK	148	32	1	3,865.91
					LAMVAFLGFVAQHAATGK	115	30	11	1,847.98
					GDAGGVVYPGGAFDPLGFAK	106	31	14	1,894.93
					NGRLAMVAFLGFVAQHAATGK	77	29	2	2,159.15
					AGNWLPGSDAPAWLPDDLPGNYGFDPLSLGK	77	32	2	3,240.56
					FTESEVIHGR	76	29	9	1,174.59
					GPIAALGEHLANPWGANFATNGISVPFF	72	31	13	2,868.45
					RFTESEVIHGR	64	31	2	1,330.69
					EIKNGRLAMVAFLGFVAQHAATGK	52	27	3	2,529.38
					EPASLKR	47	29	4	800.46
					EPASLK	37	26	2	644.36
Lhca2	tr|A8IKC8|	26,905	12	57	WAMAAVVGILFTDAVGLPK	120	28	3	1,975.07
					TGETGFLSFAPFDPMGMK	85	28	21	1,964.88
					LGVNKDNLK	72	29	14	1,000.58
					KTGETGFLSFAPFDPMGMK	67	30	2	2,092.97
					ESVPYFPWNEPWNK	67	29	2	1,792.83
					TGETGFLSFAPFDPMGMKSEEMK	61	27	2	2,585.12
					ESVPYFPWNEPWNKV	57	30	4	1,891.90
					EAELTNGR	43	27	3	890.42
					FWTAGAEK	40	26	1	909.44
					RYEIYKK	34	28	2	999.56
					LKELK	31	26	2	630.41
					RYEIYK	26	25	1	871.46
Lhca3	tr|Q75VY9|	28,870	9	136	LAMLAMLGYGAQAVMTGK	141	30	27	1,825.94
					YPGSMGQQYFLGLEAIFK	118	31	8	2,049.02
					GSGDAAYPGGPFFNLFNLGK	111	31	62	2,028.98
					WAMLGAAGCIAPEVLGAAGLIPDATNIK	89	31	5	2,796.44
					GPFQNLVEHLADPVNNNILTNFGK	83	31	24	2,651.36
					WLQYSEVIHAR	66	30	6	1,401.73
					SIAKVDR	40	29	1	788.46
					RLQDFR	32	28	1	834.45
					LQDFR	31	27	2	678.35
Lhca4	tr|Q75VZ0|	28,685	11	48	LAMLAFAGFIGQAYTTGTTPLK	121	30	7	2,288.20
					LPDGNEPGYPGGIFDPFGWSK	97	30	8	2,250.05
					DAALPSWMPGADLPGYLNGTLPGDFGFDPLYLGQDPVK	93	32	2	4,020.94
					NLSTHLADPWSTTVWQNDLAR	85	32	6	2,425.19
					YQDFVKPGSANQDPIFTNNK	78	31	11	2,283.10
					RDAALPSWMPGADLPGYLNGTLPGDFGFDPLYLGQDPVK	76	32	1	4,177.04
					WYAQAELMNAR	69	29	3	1,352.64
					RYQDFVKPGSANQDPIFTNNK	56	31	5	2,439.21
					ASAVPENVK	52	27	3	914.49
					YQDFVKPGSANQDPIFTNNKLPDGNEPGYPGGIFDPFGWSK	37	31	1	4,514.14
					LKWYAQAELMNAR	36	30	1	1,609.82
Lhca5	tr|Q75VY8|	28,211	13	120	LAMIAYMAFILQAQATGK	129	31	10	1,973.02
					GDLAGDYGWDPLGLGADPTALK	121	31	5	2,202.07
					VPNPEMGYPGGIFDPFGFSK	104	30	45	2,156.02
					NFGSVNEDPIFK	98	29	12	1,366.66
					NIGTCTVPHSVDVQGLTIPLTCLWPGSQ	97	32	4	3,050.51
					GPLAALSAHLSNPFGNNILK	95	28	19	2,034.11
					KLWAPGVVAPEYLK	85	27	5	1,570.90

					NFGSVNEDPIFKGNK	81	30	2	1,665.82
					GNKVPNPEMGYPGGIFDPFGFSK	74	31	9	2,471.17
					QSELQHAR	61	26	3	968.49
					LWAPGVVAPEYLK	54	29	4	1,442.80
					GNLKELQTK	49	29	1	1,030.59
					ELQTKEIK	29	28	1	988.56
Lhca6	tr|Q75VY6|	27,764	12	135	GDLPGDFGFDPLGLGANAESLK	146	31	16	2,190.07
					LAMLAFVGFVMAAQVTGK	118	30	7	1,885.99
					KPGSVDQDPIFSQYK	113	30	10	1,708.85
					GPIAALQEHLADPWGTTIFSK	111	30	8	2,252.17
					LPPHEVGYPGGVFAPFIPGDLAELK	93	29	56	2,620.38
					AAVVPGQAVAPPCK	84	28	10	1,364.74
					AAVVPGQAVAPPCKIPASVSYK	71	28	1	2,210.20
					ASSRPLWLPGSTPPAHLK	64	28	5	1,915.06
					GIEIPTPCFLQGLWP	60	30	5	1,727.88
					IPASVSYK	52	26	9	864.48
					ESELVHSR	47	26	7	956.47
					LPPHEVGYPGGVFAPFIPGDLAELKVK	36	27	1	2,847.54
Lhca7	tr|Q84Y02|	26,205	12	86	FAMLGAAGIILTSIGAK	140	26	7	1,649.93
					NPGSQADGSFLGFTEEFK	126	28	13	1,930.88
					LAMIACLGFAAQYAATGK	123	31	3	1,856.94
					NPGSQADGSFLGFTEEFKGLENGYPGGR	85	30	2	2,931.35
					WYDFKNPGSQADGSFLGFTEEFK	80	30	2	2,670.21
					VGLGFPEWYDAGK	75	29	5	1,438.70
					GLENGYPGGR	65	27	15	1,020.47
					FFDPMGLSR	59	27	14	1,069.51
					WYVQAELVHGR	59	30	4	1,357.70
					GPLDNLADHLADPNHVNFATNGVSIPIA	59	32	14	2,883.43
					YQEYKQK	45	26	1	986.49
					YQEYK	30	25	6	730.34
Lhca8	tr|Q75VY7|	25,904	14	140	YATGAGPVDNLAAHLK	116	30	5	1,597.83
					LAMLAFLGFVAQK	113	26	10	1,424.80
					TAMAGVAGILIPGLLTK	113	25	21	1,625.97
					KPGSQGEPGSFLGFEASLK	108	31	68	1,935.98
					GTSELGYPGGPFDPLGLSK	94	31	7	1,891.94
					DPWHVNYATNGVSLPFL	79	31	3	1,930.93
					QSWLPGSQIPAHLDTPAAQALAGNFGFDPLGLGK	79	31	4	3,474.78
					WYQQAELIHCR	68	29	5	1,503.72
					QSWLPGSQIPAHLDTPAAQALAGNFGFDPLGLGKDPVALR	67	28	3	4,126.15
					AGALNVPEWYDAGK	63	30	6	1,490.73
					YATGAGPVDNLAAHLKDPWHVNYATNGVSLPFL	50	31	3	3,508.77
					DPVALR	39	25	3	670.38
					LKEVK	35	27	1	616.40
					TAMAGVAGILIPGLLTKAGALNVPEWYDAGK	34	28	1	3,113.67
Lhca9	tr|A8ITV3|	22,827	7	108	GALAGDNGFDPLGLGQDEGR	101	30	22	1,958.92
					TQPIEGLTAHLADPFGK	101	29	6	1,794.94
					NITYYLTHLPETLGSA	91	30	61	1,792.91
					QTGTSGFINSFPFDPAGMNSPSMATK	87	29	14	2,722.21
					WAMMAVAGILGQELLGVTPAWWEAGAK	45	31	1	2,888.45
					LKWYAEAEK	36	28	1	1,137.59
					WYAEAEK	32	25	3	896.41
PsaA	sp|P12154|	83,138	15	77	DYDPTNNYNNLLDR	98	27	2	1,726.77
					AIMADLYPSFAK	86	29	5	1,342.67
					IAVDRNPVETSFEK	74	30	9	1,604.83
					EIPLPHDLLLNR	72	28	7	1,429.82
					LLDAGVDPK	61	26	5	927.51

					VAPAIQPR	58	25	4	851.50
					EILEAHR	53	25	3	867.46
					FPCDGPGR	53	25	9	905.39
					LIPDKANLGFR	52	28	1	1,243.72
					TNWGIGHSMK	49	27	4	1,130.54
					ALSITQGR	47	30	2	845.48
					ANLGFR	42	27	6	677.37
					NPVETSFEK	41	28	9	1,050.51
					WAKPGHFSR	38	27	10	1,085.56
					LKVAPAIQPR	31	25	1	1,092.69
PsaB	sp|P09144|	82,093	21	82	GGASGPVNISTSGVYQWWYTIGMR	103	31	2	2,603.23
					MQAILEAHTPPSGSLGAGHK	100	31	19	2,018.01
					DFGYSFPCDGPGR	84	25	5	1,474.61
					TPLANLVYWK	79	27	3	1,204.67
					FSQGLAQDPTTR	79	29	13	1,321.64
					IWYGLAMAHDFESHDGMTEENLYQK	76	28	5	2,985.32
					ALYGFDFLLSSK	75	30	3	1,360.71
					LFPKFSQGLAQDPTTR	71	29	3	1,805.95
					DYDPEQNKGNVLAR	69	30	2	1,618.78
					DKPVALSIVQAR	65	25	4	1,296.76
					KDFGYSFPCDGPGR	59	25	1	1,602.70
					ATKLFPKFSQGLAQDPTTR	57	29	1	2,106.13
					TNFGIGHR	52	28	3	901.46
					QILIEPVFAQWIQAAHGK	49	27	2	2,049.13
					LNHHLSGLFGVSSLAWTGHLVHVAIPESR	49	28	6	3,134.66
					FSQGLAQDPTTRR	47	31	1	1,476.76
					ATKLFPK	40	25	2	804.49
					GNVLAR	40	28	3	629.37
					DYDPEQNK	36	25	2	1,008.43
					LMPDKK	35	31	1	731.41
					GALDAR	32	29	1	602.32
PsaC	sp|Q00914|	8,842	8	39	ASQMASAPR	90	29	9	918.44
					IYDTCIGCTQCVR	88	26	5	1,645.71
					ACPLDVLEMVPWDGCK	85	28	7	1,889.86
					CETACPTDFLSVR	85	26	2	1,555.69
					VYLGSESTR	63	27	8	1,011.51
					TEDCVGCK	52	25	3	968.38
					RCETACPTDFLSVR	51	28	3	1,711.79
					TEDCVGCKR	34	25	2	1,124.48
PsaD	sp|Q39615|	21,324	17	94	EQIFEMPTGGAAIMR	121	30	8	1,650.80
					KAQTEEFYVITWEAK	114	31	6	1,842.93
					KEQIFEMPTGGAAIMR	101	31	12	1,778.89
					KEQCLALTTQLR	85	29	7	1,460.79
					AQTEEFYVITWEAK	85	29	3	1,714.83
					IGQNVNPIK	83	25	12	982.56
					KAQTEEFYVITWEAKK	78	31	1	1,971.02
					EQCLALTTQLR	75	30	7	1,332.69
					VQYLHPADGVYPEK	70	30	1	1,615.81
					VGANQNMR	67	26	16	890.41
					QGPNLLK	44	25	4	769.45
					IGQNVNPIKVK	33	25	1	1,209.73
					VFPDGK	33	28	5	662.35
					LTPCFYR	31	25	1	956.46
					FGKKEQCLALTTQLR	29	28	1	1,792.97
					IGQNVNPIKVKFSGR	28	26	2	1,656.95
					RIGQNVNPIK	28	25	7	1,138.67

PsaE	sp|P12352|	10,752	5	17	FENQNYAGVTTNNYALDEVVAAK	136	30	8	2,532.19
					YPVVVRFENQNYAGVTTNNYALDEVVAAK	111	31	1	3,244.63
					VVSVDQSGVR	79	30	2	1,045.56
					ILRPESYWFNQVGK	57	29	2	1,736.91
					YPVVVR	29	25	4	732.44
PsaF	sp|P12356|	24,040	11	105	LAWQGAGWPLAAVQELQR	117	30	30	1,994.06
					LKQYEADSAPAVALK	113	29	6	1,603.87
					GTLLEKEENITVSPR	106	29	22	1,685.91
					AGLLCGNDGLPHLIADPGLALK	89	29	12	2,215.19
					QYEADSAPAVALK	87	30	4	1,362.69
					GEAKPTDKEIIIDVPLATK	85	26	8	2,038.14
					EIIIDVPLATK	72	25	10	1,211.72
					EENITVSPR	55	29	2	1,044.53
					FANYAK	42	25	9	713.36
					QYLIAVK	40	25	1	834.50
					AYAKLEK	30	25	1	822.47
PsaG	sp|P14224|	13,118	4	16	TTGATYFDDLQK	71	28	7	1,359.64
					EANFDSTVGPK	67	28	6	1,164.55
					REANFDSTVGPK	59	30	2	1,320.65
					FVFLGYQR	35	30	1	1,029.55
PsaH	sp|P13352|	14,134	7	14	ALVALSGIAAIVTYGLK	109	25	4	1,660.00
					YFDLQDMENTTGSWDMYGVDEK	107	25	3	2,676.08
					GPQTTGENGKGGSVR	79	29	1	1,445.70
					FFTQATDIISR	74	28	2	1,298.67
					RYPDNQAK	59	29	2	991.49
					DADLPITK	44	28	1	872.47
					YGENSR	32	25	1	725.32
PsaJ	sp|P59777|	4,731	1	5	YFPDPLVFSF	51	29	5	1,231.60
PsaK	sp|P14225|	11,183	5	48	NTTAGLKLVDSK	55	27	1	1,246.70
					ADGFIGSSTNLIMVASTTATLAAARFGLAPTVK	37	29	2	3,268.72
					FGLAPTVK	36	26	2	832.49
					ADGFIGSSTNLIMVASTTATLAAAR	36	31	1	2,439.25
					NTTAGLK	32	29	1	705.37
PsaL	tr|A8IL32|	20,267	5	48	AQVISPVNGDPFVGMLETPVTSAPIVATYLSNLPAYR	142	30	18	3,905.00
					KAQVISPVNGDPFVGMLETPVTSAPIVATYLSNLPAYR	108	29	6	4,033.10
					GVEIGLAHGFLLAGPFIK	102	26	4	1,839.05
					TGVAPVLR	52	25	7	812.49
					TGVAPVLRGVEIGLAHGFLLAGPFIK	47	25	6	2,632.53
					TGVAPVLRGVEIGLAHGFLLAGPFIK	47	25	6	2,632.53
ATP synthase subunit alpha	tr|Q96550|	61,497	4	4	VVDALGQPIDGK	73	27	1	1,211.66
					HAVIIYDDLSK	63	30	1	1,273.68
					TGQIVNVPVGPGTLGR	55	25	1	1,564.88
					APGIIAR	34	25	1	697.43
Cytochrome c oxidase	tr|Q8LK22|	11,665	5	3	IPGSILGPEDAKPE	67	29	1	1,422.75
					IGEVAQR	51	29	1	772.43
					EFLEEQAK	41	28	1	993.48
					MLVDGGVEYEDGTPASASQQAK	121	29	1	2,269.03
					ALAAETEVEDGPNDEGEMFTR	111	25	1	2,296.99
Mitochondrial F1F0 ATP synthase, delta subunit	tr|A8J9X1|	21,154	2	2	SALAAANSAQGQGDEYDQAANR	127	28	1	2,207.99
					AALELYAALDSALDQK	44	30	1	1,691.89
Mitochondrial processing peptidase alpha subunit	tr|A8IKI9|	49,571	6	9	LNQLEALEILADAVVNAR	89	27	4	1,952.08
					GVTDVQLATAK	68	29	1	1,102.61
					DHVAFGVDATR	50	28	1	1,187.58
					GTGLVGVVASGAPGK	48	28	1	1,269.72
					TSTPATKPIVQTSSLR	43	28	1	1,686.94

Predicted protein	tr|A8IHW6|	27,874	2	2	EGLEAANQVAAEGK	53	29	1	1,386.69
					MVAAEEEYVR	35	26	1	1,212.56
Triose phosphate translocator	tr|A8HN02|	42,975	2	2	ELDALETTVSK	73	29	1	1,205.63
					KAPPPPAAAGGASPAAA	39	31	1	1,401.75
Cytochrome c oxidoreductase 50 kDa subunit	tr|A8J5P7|	55,037	7		LASDAFGSVPDEDAATSVR	114	30	1	1,907.90
					AVNILSDILLNSNLDAR	102	28	1	1,841.01
					IDAVDANAIR	69	29	1	1,057.56
					MVLAAAGAVNHDELVK	57	29	1	1,653.86
					FTGSYVHDR	46	27	1	1,081.51
					DVVATDANPFLR	44	31	1	1,317.68
					FSNPRPSPIDHTPLLSTLPETR	37	30	1	2,475.30
Cytochrome c oxidoreductase 7 kDa subunit	tr|A8JC51|	7,015	3	3	LFSDIIAER	76	27	1	1,063.58
					LSEALYQTFFK	51	30	1	1,346.70
					LFSDIIAERT	31	28	1	1,164.63
Trypsin	sp|P00761|	24,391	3	12	LGEHNIDVLEGNEQFINAAK	136	31	3	2,211.10
					VATVSLPR	60	28	6	842.50
					LSSPATLNSR	53	30	3	1,045.56
keratin 1, type II, cytoskeletal - human	KRHU2	65,477	20	25	FLEQQNQVLQTK	93	30	1	1,475.79
					SKAEAESLYQSK	83	29	2	1,340.67
					LNDLEDALQQAK	74	30	1	1,357.70
					YEELQITAGR	68	29	2	1,179.60
					TNAENEFVTIKK	67	29	2	1,393.73
					TNAENEFVTIK	64	29	1	1,265.64
					NKLNDLEDALQQAK	61	30	1	1,599.83
					THNLEPYFESFINNLR	60	31	1	1,993.98
					AEAESLYQSK	59	27	1	1,125.54
					TLLEGEESR	45	29	1	1,033.52
					NMQDMVEDYR	44	25	1	1,332.52
					DVDGAYMTK	43	25	2	999.44
					MSGECAPNVSVTVSTSHTSISGGGSR	42	29	2	2,581.16
					LRSEIDNVKK	39	28	2	1,201.69
					SEIDNVKK	37	29	1	932.50
					DYQELMNTK	37	25	2	1,157.51
					NKYEDEINKR	37	29	1	1,308.65
					YEDEINKR	35	28	2	1,066.52
Keratin, type I cytoskeletal 10	K1C10	59,502	12	14	GSLGGGFSSGGFSGGSFSR	105	28	1	1,707.77
					QSLEASLAETEGR	97	30	1	1,390.68
					ALEESNYELEGK	91	28	1	1,381.65
					AETECQNTEYQQLLDIK	83	29	1	2,082.97
					SLLEGEGSSGGGGR	80	29	1	1,262.60
					SGGGGGGGGCGGGGGVSSLR	66	26	1	1,549.68
					SQYEQLAEQNR	54	29	1	1,365.64
					ADLEMQIESLTEELAYLK	54	31	2	2,112.04
					SQYEQLAEQNRK	52	30	1	1,493.73
					LKYENEVALR	44	27	1	1,234.68
					VTMQNLNDR	41	28	2	1,106.53
					LENEIQTYR	34	29	1	1,165.58
Keratin, type I cytoskeletal 9	K1C9	62,113	16	18	GGSGGSHGGGSGFGGESGGSYGGGEEASGSGGGYGGGSGK	152	25	1	3,223.28
					GGSGGSYGGGGSGGGYGGGSGSR	137	25	1	1,791.73
					GGGGSFGYSYGGGSGGGFSASSLGGGFGGGSR	128	26	2	2,705.16
					SGGGGGGGLGSGGSIR	127	29	1	1,232.60
					VQALEEANNDLENK	108	29	1	1,586.77
					VQALEEANNDLENK	106	29	1	1,586.77
					DIENQYETQITQIEHEVSSSGQEVQSSAK	105	31	1	3,264.51
					HGVQELEIELQSQLSK	100	30	2	1,837.97

					FSSSSGYGGGSSR	93	25	1	1,235.53
					EIETYHNLLEGGQEDFESSGAGK	84	29	1	2,510.13
					QGVDADINGLR	68	28	1	1,157.59
					STMQELNSR	68	27	2	1,065.50
					QVLDNLTMEK	65	30	1	1,206.60
					YCGQLQMIQEQISNLEAQITDVR	50	31	1	2,753.32
					KGPAAIQK	50	25	1	812.49
					TLLDIDNTR	46	30	1	1,060.56
Keratin, type II cytoskeletal 2 epidermal	K22E	65,848	10	11	GFSSGSAVVSGGSR	99	27	1	1,254.61
					GSSSGGGYSSGSSSYGSGGR	83	25	1	1,740.71
					GGSISGGGYGSGGGK	68	27	1	1,197.55
					NVQDAIADAEQR	57	28	1	1,329.64
					NLDLDSIIAEVK	56	30	1	1,329.73
					KYEDEINKR	54	28	1	1,194.61
					STSSFSCLSR	50	25	1	1,131.51
					LQGEIAHVK	45	25	2	994.56
					AQYEEIAQR	41	29	1	1,107.54
					YLDGLTAER	40	28	1	1,037.53

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